RECENT STUDIES ON ALBOMYCIN, A NEW ANTIBIOTIC

BY

G. F. GAUSE, D.Biol.Sc.

Deputy Director, Institute for Antibiotic Research of the Academy of Medical Sciences of the U.S.S.R., Moscow

Albomycin, a new antibiotic, has been manufactured during recent years by the pharmaceutical industry of the Soviet Union. It has been studied both in the laboratory and in clinical practice, and it is the purpose of this paper to summarize the results of the most important studies.

Origin and Chemical Nature

Albomycin was obtained by Gause and Brazhnikova (1951) from cultures of a new species of streptomycetes, *Actinomyces subtropicus*. This antibiotic strongly inhibits the growth of Gram-positive cocci, chiefly pneumococcus and staphylococcus. More important, it inhibits the growth of staphylococci resistant to other antibiotics, including penicillin, streptomycin, the tetracyclines, and erythromycin. It is also effective against a number of Gram-negative bacteria—for instance, the coli-dysentery group of organisms and Friedländer's bacillus. Pure albomycin inhibits the growth of staphylococci in a dilution of 1 in 700 millions, whereas crystalline penicillin inhibits their growth in a dilution of 1 in 80 millions. In other words, 1 mg. of albomycin inhibits the growth of staphylococci about ten times more strongly than an equal amount of penicillin. Albomycin was found to be practically non-toxic for animals and in clinical practice, and it was therefore studied in greater detail.

Data on the chemical nature of albomycin were published by Brazhnikova, Lomakina, and Muravieva (1954). The isolation of chemically pure albomycin from the culture fluid of *Actinomyces subtropicus* presents difficulties which can be overcome in a number of ways. Albomycin is a basic substance and forms salts with various acids. Chemically pure sulphate of albomycin is in the form of an amorphous red powder, easily soluble in water, slightly soluble in methanol, but insoluble in other organic solvents.

The pure preparation of albomycin sulphate contains 4.16% of iron. Experiments have shown that it is possible to remove iron from the molecule of albomycin by treating it with acetone containing hydrochloric, hydrobromic, or hydriodic acid or with oxychinolin. Under this treatment the bright orange colour of albomycin solutions disappears and their antibacterial activity is reduced 14 to 15 times. When, however, a drop of 5% solution of ferric chloride is added to the watery solution of the inactivated albomycin, both colour and antibacterial activity are completely restored. Hence the iron content of albomycin represents a labile functional group, which can be split off in acetone media in the presence of hydrohalogen acids. When albomycin solution in acetone is acidified with sulphuric or nitric acid, or if ethanol is used instead of acetone, the splitting of iron is not observed. It seems probable that the iron is attached by a chelate bound to the hydroxy group of serine, which is contained in the molecule of albomycin.

Pure albomycin gives a positive biuret reaction; it contains 13.28% of total nitrogen (Kjeldahl) and 1.34% of amino-nitrogen (Van Slyke). During the first five minutes 1.22% of amino-nitrogen can be determined, and during the next 25 minutes only 0.12%. When albomycin is subjected to hydrolysis by hydrochloric acid for 26 hours the amount of amino-nitrogen increases from 1.34% to 7.2%. Hydrolysate of pure albomycin contains a number of amino-acids; therefore this antibiotic may be said to belong to the class of metal-containing peptides.

From the fact that pure albomycin gives a negative reaction with ninhydrin, and that this becomes positive only after slight heating with dilute hydrochloric acid, it can be concluded that albomycin does not contain free α-amino groups, the latter appearing only after hydrolysis. Albomycin appears to be a cyclopeptide. It has been shown that it contains a free amino group which participates in the formation of salts. When albomycin is deaminated under mild conditions its antibacterial activity is completely lost.

If the equivalent weight of albomycin is calculated on the assumption that the molecule of this antibiotic contains one atom of iron, one residue of sulphuric acid, and one amino group, the values 1346, 1336, and 1270 are obtained. This indicates that the molecular weight of albomycin is not less than 1300.

TABLE I.—*Amino-acid Composition of Albomycin*

Rf	Butanol–Water–Acetic Acid		Water-saturated Phenol–Ammonia	
	Pure Amino-acid	Albomycin Hydrolysate	Pure Amino-acid	Albomycin Hydrolysate
Ornithine	0·12	0·12	—	—
Serine	0·20	0·194	0·34	0·34
Glutamic acid	0·23	0·24	—	—
Alanine	0·273	0·288	0·57	0·57
Proline	0·35	0·36	0·81	0·81
Glycine	0·25	0·26	0·35	0·37

TABLE II.—*Amino-acid Composition of Albomycin (Two-dimensional Chromatograms)*

Rf Butanol / Rf Phenol	Albomycin Hydrolysate	Pure Amino-acid
Ornithine	0·21	0·18
Serine	0·62	0·61
Glutamic acid	0·20	1·20
Alanine	0·60	0·59
Proline	0·45	0·45
Glycine	0·70	0·71

Chromatographic studies (Tables I and II; Fig. 1) have shown that the hydrolysates of albomycin contain seven amino-acids. Six of these have been identified with ornithine, serine, glutamic acid, alanine, glycine, and proline; the seventh has not so far been identified with any of the known amino-acids. The amount of serine and ornithine in the molecule of albomycin is greater than that of the other amino-acids.

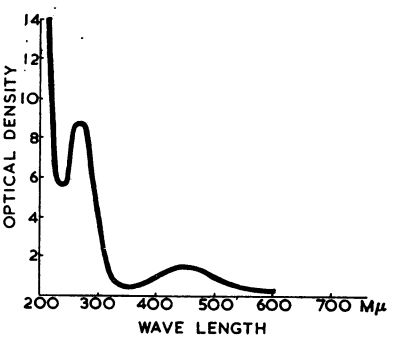

FIG. 1.—Absorption spectrum of albomycin.

It can therefore be concluded that albomycin differs significantly in chemical structure from the other antibiotics so far described; it also differs markedly from griseine, the only iron-containing antibiotic produced by an actinomycete.

Mechanism of Antibacterial Action

Study of the sensitivity to albomycin of the commoner bacteria has shown that the following are sensitive: staphylococci, *Escherichia coli, Aerobacter aerogenes, Sarcina subflava,* and *Bacillus subtilis* (Gause and Brazhnikova, 1951). Further, pneumococci, *Klebsiella,* dysentery bacilli, and some (but not all) strains of *Streptococcus pyogenes* are very sensitive. Somewhat less sensitive are meningococci and *Haemophilus pertussis* (Shorin, 1951). Albomycin strongly inhibits *Spirochaeta sogdianum in vivo.* The following are insensitive to albomycin: *Listerella,* tubercle bacilli, and *Bacillus mycoides.*

An analysis was also made of the incidence of albomycin-resistant strains of staphylococci in various infections in

clinical practice. It was found that about 31% of strains are resistant to penicillin, and about 1% resistant to albomycin. Strains resistant to albomycin were found to be penicillin-sensitive (Shorin, 1951).

The mechanism of the antibacterial action of albomycin is interesting. Its action on the respiration of staphylococci and *E. coli* has been shown to be a typically bacteriostatic one (Sazykin, 1955). It was observed by Shorin and Sazykin (1954) that albomycin, independently of concentration, inhibits the growth of staphylococci, *E. coli*, and other bacteria only in the presence of oxygen. In these experiments narrow glass tubes containing agar nutritive medium were before inoculation placed for 20–30 minutes in a boiling water-bath to eliminate air from the agar, and afterwards rapidly cooled to 45°–50° C. At this temperature two or three drops of bacterial suspension were put into each test-tube together with the necessary amount of albomycin dissolved in 0.1 ml. of sterile distilled water. The medium in the test-tubes was then carefully mixed by shaking, precautions being taken to avoid air bubbles forming in the agar. Afterwards the tubes were cooled until the agar solidified and then incubated at 37° C. for 24 hours.

It was observed that albomycin inhibits the growth of bacteria only in the upper layer of the agar medium—that is, under aerobic conditions. In the bottom layer of the agar in the narrow test-tubes, where the oxygen does not penetrate, the growth of bacteria is not inhibited by any concentration of albomycin up to 5,000 units per ml., the greatest concentration tested (Fig. 2).

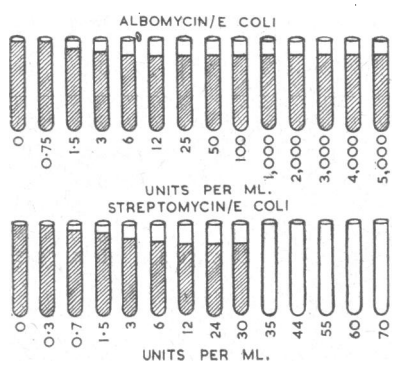

FIG. 2.—Effect of albomycin and of streptomycin on growth of *E. coli* in nutritive agar.

That albomycin inhibits the growth of sensitive bacteria only in the presence of oxygen leads to the conclusion that it is active only when the bacterium is breathing with the aid of the oxygen-transporting enzyme, which, as is well known, contains iron in its molecule. The hypothesis may be advanced that the structure of albomycin is similar to that of some component of the iron-containing respiratory system of the bacterial cell, and hence that it impedes the working of this system and interferes with the growth of the bacteria. In the absence of oxygen the respiration of the sensitive bacteria takes some other course; the iron-containing oxygen-transporting enzyme is no longer involved in the process of respiration, and it is no longer possible for the albomycin to exert its antibacterial action.

It is to be expected that increasing the concentration of iron in the nutritive medium of staphylococci growing in Petri dishes will increase the concentration of the respiratory enzyme in their cells, and in this way decrease the action of albomycin on staphylococci as measured by the agar diffusion method. It was actually observed by Brazhnikova that by adding 0.04% of ferric chloride to the nutritive-agar medium upon which staphylococci are growing the action of albomycin upon these organisms is decreased by about 30%.

On the basis of the above data the molecule of albomycin may be considered as an "imitation" of some iron-containing respiratory enzyme of the sensitive bacteria, in which iron functions as a prosthetic group and the polypeptide part as a protein carrier. This being so, further investigation of the structure of albomycin and of the type of binding of iron with the polypeptide rest of the molecule could be of immediate theoretical interest for the study of the respiratory enzymes and for the understanding of the process of respiration in bacteria.

Pharmacological Properties

The absence of toxicity of albomycin has been demonstrated both experimentally in animals and clinically. It is impossible to determine the lethal dose of the antibiotic for mice, rabbits, cats, or guinea-pigs. It is well tolerated when injected subcutaneously or intravenously in the maximum concentrations tested—that is, 50 million units per kg. of body weight (mice). As regards absence of toxicity albomycin can be compared only with penicillin. It is interesting to note that the drug is also well tolerated by guinea-pigs, for which penicillin is rather toxic.

Albomycin is pharmacologically inactive (Shorin, 1951); intravenous injections of large doses in animals do not affect the heart, blood pressure, or respiration. It is devoid of cumulative toxicity and pyrogenic action, and no local reactions are observed when it is injected subcutaneously or intramuscularly in animals or man. The intrathecal injection of albomycin is safe and not accompanied by any side-reaction. Histological examination of organs and tissues of laboratory animals given daily injections of large doses of albomycin for a considerable period (up to 30 days) did not reveal any deviations from the normal. The composition of blood and the activity of leucocytes are also not affected by albomycin (Rossolimo, 1951). Complete absence of toxicity of the drug for man is also proved by extensive clinical practice over several years.

A remarkable feature is the formation of a reversible complex between albomycin and serum proteins, which is of importance for its circulation in the body (Gause and Brazhnikova, 1951; Judinzev, 1951). Experiments have shown that its antibacterial action is not decreased when albomycin is used in 1% solution of casein or 10% solution of egg albumen. However, in the presence of 10% of blood serum, and particularly in whole serum, the antibacterial action of albomycin is reduced. This effect results from the partial and reversible binding of albomycin with the serum proteins. It is impossible to detect the presence of albomycin by the usual method of titration of this antibiotic with sensitive bacteria if its concentration in the serum attains 150 units per ml. or less. A greater concentration can be detected by the method of biological titration. Albomycin freely passes through collodion membranes, and experiments on its dialysis from solution in serum across such membranes have shown that the amount of antibiotic bound with proteins, and therefore non-dialysable, attains 100–200 units per ml. of horse serum, regardless of the amount of albomycin added to the serum.

The presence of active albomycin in the blood serum can be demonstrated in the following way. It has already been mentioned that if the concentration of albomycin attains 100 units per ml. of serum the latter is completely "inactive" so far as its antibacterial action is concerned. When, however, serum proteins containing "inactive" albomycin are sedimented by trichloracetic acid, the sediment removed by filtration, and the supernatant liquid neutralized, this liquid will retain in active form most of the albomycin which was initially added. In this way the antibacterial action of albomycin, at first "masked" by the serum, can be completely restored after sedimentation of the serum proteins (Gause and Brazhnikova, 1951). The method of estimating the concentration of albomycin in the blood serum is based on this principle. It is interesting that the complex of albomycin with serum proteins, if injected intraperitoneally into mice infected with pneumococci or dysentery bacilli, possesses chemotherapeutic activity. This experiment shows that the complex under investigation, inactive against bacteria *in vitro*, is capable of dissociation *in vivo* with reappearance of the antibiotic in the active form (Shorin, 1951).

The experiments above outlined are important for explaining the specific features of the circulation of albomycin in the body. It was observed by Judinzev (1951) in experiments with rabbits that after the first subcutaneous injection the amount of albomycin excreted by the kidney was not

great, because it is partially bound with proteins. After repeated injection the amount of albomycin excreted by the kidney increases sharply, since the serum proteins are already saturated with the antibiotic and the latter appears in the form of a "free" compound. One unit of albomycin inhibits the growth of staphylococci in 1 ml. of nutritive agar, and is approximately equal to 1/50 unit of penicillin. After subcutaneous injection into a rabbit of 100,000 units per kg. body weight albomycin circulates in the blood for three days. The maximum concentration of albomycin in the blood (240 units per ml.) is attained within 30 minutes of subcutaneous injection. Thereafter the concentration gradually decreases, and at the end of the fourth hour reaches 150 units per ml., and at 24 hours 100 units per ml. (Fig. 3). Albomycin remaining free and not bound by serum proteins is usually excreted in the urine for six to eight hours after injection (usually 70 to 80% of the injected dose), while the protein-bound albomycin is slowly excreted from the organism for two or three days. Albomycin can be detected in the lymph, and penetrates well into various organs and tissues; it is, however, unable to pass into the cerebrospinal fluid (Judinzev, 1954).

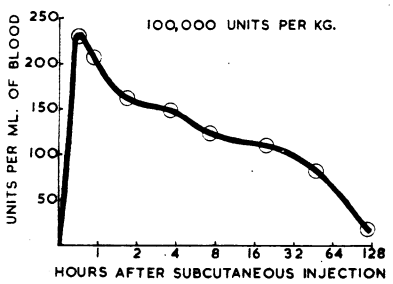

FIG. 3.—Concentration of albomycin in blood of rabbit after single subcutaneous injection of albomycin in a concentration of 100,000 units per kg.

Chemotherapeutic Investigations

It has been shown by Shorin (1951) in experiments in mice infected with 500,000 lethal doses of pneumococci that 100% survival can be obtained with a single subcutaneous injection of 750,000 units of albomycin per kg.; in parallel experiments with penicillin only 20% survival was observed. In experiments in mice infected with various strains of staphylococci and haemolytic streptococci injection of albomycin invariably yielded positive therapeutic results. In mice infected with virulent strains of dysentery bacilli a single injection of albomycin had the same effect as injection of an equal amount of streptomycin. In guinea-pigs infected with *Sp. sogdianum* both the blood and the brain became sterile after treatment with albomycin in a dosage of 150,000 units per kg. daily for 14 days. When equal amounts of penicillin were used (in dilution units = 1/50 of Oxford units) the results were manifestly inferior. In mice infected with 50 lethal doses of Friedländer's bacillus a single subcutaneous injection of 25,000 units of albomycin per kg. led to 100% survival; when the number of lethal doses was increased to 500 the amount of albomycin required to attain the same effect was increased four times —that is, to 100,000 units per kg.

In experimental infection of animals with tubercle bacilli, *Listerella, Salmonella,* and *Rickettsia,* albomycin was found to be ineffective.

The action of albomycin combined with other antibiotics was studied in experiments in mice infected with pneumococci, it being relatively easy in these circumstances to vary the number of lethal doses and to attain quantitative results. It was recorded that the action of albomycin *in vivo* in combination with penicillin or with streptomycin is clearly synergistic.

Clinical Application

In clinical practice albomycin is used mainly in the treatment of various infections caused by pathogenic cocci. In paediatrics it is used in the treatment of pneumonia, particularly in young children. Positive therapeutic results were obtained in the septic complications of dysentery and measles and in various septic conditions caused by penicillin-resistant bacteria (Sokolova and Smirnova, 1953; Dobrochotova, 1951; Krechmer *et al.,* 1951; Raicher, 1952; and Shapiro, 1951). Intrathecal injections of albomycin have given good results in the treatment of meningitis caused by penicillin-resistant pneumococci. No side-reactions were recorded after intrathecal injections of 100,000–200,000 units of albomycin in children. Gilevich (1953) has shown albomycin to be very effective in the treatment of relapsing fever due to *Sp. sogdianum,* the dosage for adults being 3 million units intramuscularly twice a day for 7–12 days.

Zemskov (1953) and Selzovsly have demonstrated the activity of albomycin in the treatment of peritonitis and other surgical infections. Berent and Gilman (1954) used albomycin to treat prostatitis and gonococcal urethritis caused by penicillin-resistant bacteria. As is evident from the number of papers published, the use of albomycin in clinical practice is gradually extending.

Summary

Results of laboratory and clinical investigations of albomycin, a new antibiotic prepared in the U.S.S.R. from *Actinomyces subtropicus,* are presented.

Albomycin is effective against a variety of organisms, and particularly against staphylococci resistant to other antibiotics. Its action is about ten times as strong as that of penicillin.

Its chemical nature and pharmacology are discussed.

It forms a reversible complex with the serum proteins, which facilitates its circulation in the body. It is non-toxic, and well tolerated in large doses—up to 50 million units per kg.—given subcutaneously or intravenously. No side-reactions were noted after intrathecal injections in children.

Albomycin has proved effective in the treatment of pneumonia, especially in young children, in the septic complications of dysentery and measles, and in meningitis due to penicillin-resistant pneumococci. It has also been used in the treatment of peritonitis and other surgical infections, and for penicillin-resistant prostatitis and gonococcal urethritis.

REFERENCES

Berent, I. E., and Gilman, K. S. (1954). *Sovetsk. Med.,* No. 7, 34.
Brazhnikova, M. G., Lomakina, N. N., and Muravieva, L. I. (1954). *Compt. rend. Acad. Sci. U.R.S.S.,* **99**, 827.
Dobrochotova, A. I. (1951). *Nov. Med. (Mosk.),* **23**, 35.
Gause, G. F., and Brazhnikova, M. G. (1951). Ibid., **23**, 3.
Gilevich, Y. S. (1953). *Med. Parazit.,* **5**, 433.
Judinzev, S. D. (1951). *Nov. Med. (Mosk.),* **23**, 22.
—— (1954). *Compt. rend. Acad. Sci. U.R.S.S.,* **99**, 115.
Krechmer, B. B., *et al.* (1951). *Sovetsk. Med.,* No. 10, 10.
Raicher, E. A. (1952). Ibid., No. 11, 18.
Rossolimo, O. K. (1951). *Nov. Med. (Mosk.),* **23**, 30.
Sazykin, Y. O. (1955). *Mikrobiologija,* **24**, 75.
Shapiro, S. L. (1951). *Nov. Med. (Mosk.),* **23**, 53.
Shorin, V. A. (1951). Ibid., **23**, 7.
—— and Sazykin, Y. O. (1954). *Compt. rend. Acad. Sci. U.R.S.S.,* **96**, 645.
Sokolova, K. F., and Smirnova, M. K. (1953). *Pediatrija,* No. 1, 23.
Zemskov, N. N. (1953). *Vrac. Delo,* **5**, 425.

To allow blind physiotherapists to give ultraviolet light therapy with the same accuracy and confidence as their sighted colleagues an electrical apparatus has been evolved, the "erythema meter," which measures the degree of redness of the patient's skin. Essentially it consists of a photoelectric detector which converts colour impressions to sound signals; thus a blind operator can tell, by comparing control non-irradiated skin with the irradiated area, whether his patient is in danger of receiving too heavy a dose. The "erythema meter," which is quite small and compact, was designed and made by Electro-Medical Supplies (Greenham), Ltd., in conjunction with the Royal National Institute for the Blind, the Institute also providing a research grant for the work. The apparatus has been approved by a panel of the Chartered Society of Physiotherapy, and steps are now being taken to train blind physiotherapists in its use.